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(71) Applicants (*for all designated States except US*): **LIFE-POINT, INC.** [US/US]; 10400 Trademark Street, Rancho Cucamonga, CA 91730 (US). **ADVANCED EXTRAVASCULAR SYSTEMS** [US/US]; 1551 Westwood Boulevard, Suite 110, Los Angeles, CA 90024 (US).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **LIANG, Greg** [CN/US]; 10400 Trademark Street, Rancho Cucamonga, CA 91730 (US). **FOLEY, Thomas** [IE/US]; 10400 Trademark Street, Rancho Cucamonga, CA 91730 (US).

(74) Agent: **LYON & LYON LLP**; KWASIGROCH, Lois, M., 633 West Fifth Street, Suite 4700, Los Angeles, CA 90071-2066 (US).

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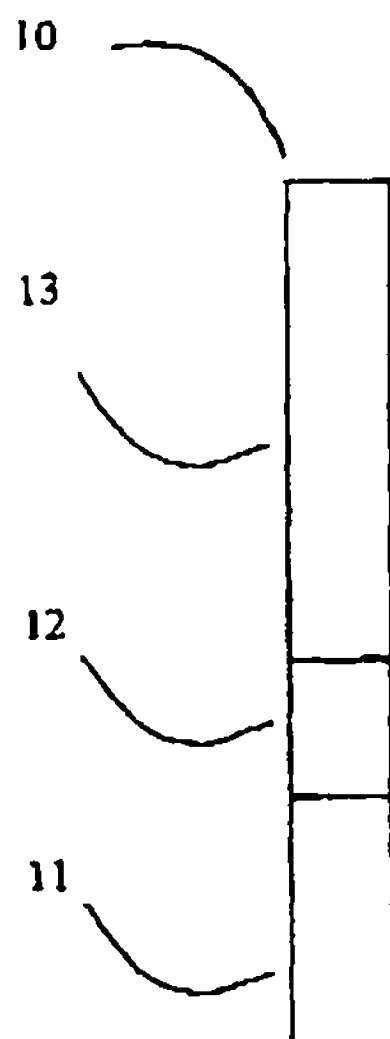
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(54) Title: ASSAY METHOD AND DEVICE FOR DETECTING THE PRESENCE AND CONCENTRATION OF ANALYTES WHILE SIMULTANEOUSLY PREVENTING ANALYTE ADSORPTION TO SOLID MATERIALS USED IN THE ASSAY



(57) Abstract: This invention provides a method and a device for determining the presence and/or quantity of a hydrophobic analyte in a biological solution suspected of containing the analyte with enhanced sensitivity and precision. Sensitivity and precision of the method are achieved by selecting an antibody capable of binding to hydrophobic regions of the analyte, adding the selected antibody to the analyte before applying the solution containing the analyte to solid materials of the method and device, and hence, preventing adsorption of the analytes to the solid materials.

WO 02/14869 A2

DESCRIPTIONAssay Method And Device For Detecting The Presence And
Concentration Of Analytes While Simultaneously Preventing
Analyte Adsorption To Solid Materials Used In The Assay5 1. Field Of The Invention

This invention relates to chemical analysis tests, and in particular, to a chromatographic assay device and methods for detecting the presence and/or concentration of various analytes in bodily fluids, such as saliva. The assay device and the methods described in this disclosure allow increased sensitivity of results by preventing analyte loss through analyte adsorption to solid materials used in the assay.

2. Background

Chromatographic immunoassay with visual read-out has often been used in detecting many analytes. See U. S. Patent Nos. 5,559,041; 4,943,522; 5,602,040; 5,559,645, 5,037,736 and 4,098,876, and Wheeler, Prof. Nurse 14:571 (1999), which are incorporated herein by reference, as if fully set forth. In a typical chromatographic immunoassay, a sample containing the analyte to be tested is added to a solid support. The solid support contains a movable labeled specific antibody to the analyte, as well as an immobilized binding reagent. Upon addition to the solid support, the sample containing the analyte migrates to the zone containing the movable labeled specific antibody. There, the analyte binds to the antibody forming a labeled antibody-analyte complex. Next, the labeled antibody-analyte complex migrates to the zone containing the immobilized binding reagent. Generally, with chromatographic sandwich assays, the immobilized binding reagent is an antibody to the analyte and is capable of capturing the labeled antibody-analyte complex to form a labeled antibody-analyte-immobilized antibody sandwich. On

the other hand, with competitive chromatographic immunoassays, the immobilized binding reagent is a binding pair of the labeled antibody. This binding pair binds to only labeled antibodies that are not bound by the analyte.

5 In other words, the binding pair of the labeled antibody binds to unbound free labeled antibodies. Once binding is complete, any unbound labeled antibody or labeled antibody-analyte complex migrates further down the chromatographic unit past the immobilized binding reagent zone. Detection

10 of the label within the immobilized binding reagent zone shows the presence and or/quantity of the particular analyte in the sample solution. See Dresser, D. W. Handbook of Experimental Immunology, 1: 8.1-8.21, (ed. Weir, D. M. 1986), Hall, B. J. et al., Analyt. Chem., 70: 1788-1796

15 (1999), Hurn, B. A. L. and Chantler, S.M., Meth. Enzymol., 70: 104-41 (1980), Kiyai, K., Principles and Practice of Immunoassay, 246-264 (eds. Price and Newman), (M. Stockton Press, NY, 1991), Seabrook, R. and Atkimon, T, Principles and Practice of Immunoassay, (eds. Price and Newman), (M.

20 Stockton Press, NY, 1991), Roth, K. D. W. et al., J. Analytical Toxicology, 20: 291-300 (1996), Playfair, J. H. L. et. al., Brit. Med. Bull., 30: 24-31 (1974), which are incorporated herein by reference as if fully set forth.

Chromatographic immunoassays with visual read-out have

25 the advantage of being instrument free and rapid in terms of obtaining assay results. It is because of this very recognized advantage that a number of chromatographic assay devices have become available for use over-the-counter (OTC). For instance, the HCG test for pregnancy is commonly

30 available to consumers over the counter. See Ekins, R.P. (1976), Hormone Assays and their Clinical Application. 4th edition (eds. Galfre, G. and Milstein, C , and Meth. Enzymol., 73:3-48 (1981). Furthermore, many chromatographic immunoassay devices are being used for point-of-care (POC)

35 testing. Such chromatographic immunoassay POC tests include the test for streptococcus and the one for

tetrahydrocannabinol (THC), the active component of marijuana.

Most chromatographic immunoassay devices use porous materials at the sample addition area. These materials
5 cause some of the analyte to become adsorbed to the surface of these porous materials before the analyte contacts the assay reagents of the device. This adsorption often results in poor assay sensitivity.

For instance, a number of POC tests for detecting THC
10 constitute chromatographic immunoassay devices that use porous materials at the sample addition area. The THC-analyte is adsorbed onto the porous surface before it contacts the assay reagent. As such, most of the available POC tests for THC have poor assay sensitivity and precision.

15 Summary Of The Invention

This invention involves a method and device for prevention of loss of analytes through adsorption to solid materials used in the analysis by adding an antibody to a solution containing an analyte which will bind with the
20 analyte, and then applying the analyte-antibody complex in the solution to a solid support. The solid support of the assay consists of a separate chromatography unit having a sample addition zone, a test zone, and an absorption zone. In general, a sample mixed with a first antibody is added to
25 the sample addition zone, from where it migrates via capillary flow onto the test zone. The test zone contains either an immobilized analyte or analog of the analyte or an immobilized second antibody. The immobilized analyte or analog of the analyte binds to unbound labeled antibody,
30 whereas the immobilized second antibody binds to the first-antibody-analyte complex in the solution. Any labeled antibody that is not bound at the immobilized antibody or immobilized antigen zone flows through onto the absorption zone.

Adding the antibody capable of binding to the hydrophobic regions of the analyte in the sample containing the analyte prior to applying the sample solution to the chromatography unit decreases adsorption of the analyte to the solid materials of the chromatography unit. This increases sensitivity and enhances precision of the assay measurement.

Brief Description Of The Drawings

Figure 1 shows a chromatographic assay strip 10 having a sample addition zone 11, a test zone 12, and a receiving zone 13.

Figure 2 shows a chromatography assay strip 20 having a sample addition zone 21, a test zone 22, a receiving zone 23, and an antibody labeling zone 24. An antibody labeling reagent is supported at zone 24.

Figure 3 depicts a container for the solubilizing reagent.

Detailed Description Of The Preferred Embodiments

The assay materials of the invention comprise a chromatographic assay device and a solubilizing reagent containing an antibody specific for the analyte.

The chromatographic assay device of the invention comprises a porous chromatographic strip with an upstream end and a downstream end. At the upstream end of the porous strip is a sample addition zone for receiving a sample solution. A test zone having an immobilized binding reagent is disposed downstream of the sample addition zone. In addition to the test zone toward the downstream end of the assay strip there is an absorption zone.

All zones of the porous strip are physically in flow communication so that a sample solution applied to the sample addition zone will flow through the test zone until it reaches the absorption zone.

The materials of the zones of the assay strip are chosen from a group of materials that includes cellulose, fiberglass, nylon, polyester, etc. The material for the sample addition zone must be capable of receiving a sufficient amount of sample solution. Fiberglass, polyester, and cellulose paper are among the preferred materials for the sample addition zone. The material for the test zone must have the capability of immobilizing a minimum of $10\mu\text{g}/\text{cm}^2$ of protein. Nitrocellulose membrane is one of the preferred materials for the test zone. The material for the absorption zone is capable of receiving the sample solution with all unbound reagents at the test zone. The preferred materials for the absorption zone and the sample addition zone include cellulose paper.

The antibody of the solubilizing reagent is capable of binding to the analyte molecule and blocking the hydrophobic regions of the analyte, thus preventing the analyte from being adsorbed to the surface of the sample addition zone of the chromatographic assay strip. Many of the regions where the antibody binds the analyte molecule are hydrophobic. Without these hydrophobic regions of the analyte being bound, the analyte would be more likely to be adsorbed to hydrophobic regions of the chromatography strip. Such adsorption would decrease the amount of analyte reaching the test zone and reduce sensitivity and accuracy of the assay.

In one preferred embodiment of the invention, the assay device and the solubilizing reagent are for a competitive chromatographic immunoassay. The solubilizing reagent consists of a labeled antibody. The binding reagent at the test zone consists of the analyte or analogues of the analyte, which are capable of binding antibodies to the analyte. For example, a preferred binding reagent for a THC test is THC-bovine serum albumin (BSA) conjugate. The binding reagent is immobilized at the test zone by adsorption or covalent binding. Nitrocellulose membrane is

capable of binding large concentration of proteins by passive adsorption.

In a different embodiment of the invention, which is another competitive assay, the solublizing reagent consists of an unlabeled antibody. The binding reagent at the test zone consists of the analyte or an analogue of the analyte, capable of binding antibodies to the analyte. The solid support or chromatographic unit of this assay device further comprises an antibody-labeling zone having an antibody-labeling reagent. The antibody-labeling zone is positioned downstream from the sample addition zone and upstream from the test zone. A labeling reagent capable of binding the antibody of the analyte is movably supported at the antibody-labeling zone. The labeling reagent is a binding protein of the antibody conjugated with a reporter substance (label). The binding protein of the antibody is chosen from a group of proteins including antibodies, protein A, and protein G. The label of the labeling reagent is chosen from a group of fluorescent, chemiluminescent, bioluminescent compounds, radioactive isotopes, enzymes, and particular color particles. Such particular particles include colloidal gold, colloidal silver, carbon black, latex beads, etc., which can be seen by the naked eye. The label is coupled to a binding protein via various means including covalent binding and physical adsorption, which have been described in previous publications. See U.S. Patent Nos. 5,252,496, Kang et al.; 4,313,734, Leuvering, et al.; Hermanson, G., *Bioconjugate Techniques*, Academic Press, San Diego (1996), which are incorporated herein by reference as if fully set forth. The solubilizing reagent to be used with a device having an antibody-labeling zone consists of a specific antibody of the analyte, which antibody can be labeled by the labeling reagent at the antibody-labeling zone.

In a different embodiment of the invention, the assay device and reagent are for a sandwich chromatographic

immunoassay. The binding reagent at the test zone is an antibody of the analyte. The solubilizing assay reagent consists of a labeled antibody that recognizes a different epitope of the analyte than the antibody of the binding reagent. Thus, both antibodies can bind the same analyte to form a labeled antibody-analyte-binding antibody "sandwich".

In accordance with the present invention, a sample solution containing the analyte is first exposed to the solubilizing reagent. If the analyte is present in the sample solution, the analyte binds to the antibody in the solubilizing reagent to form an antibody-analyte complex. The resulting solution is then applied to the sample addition zone of the assay device. The labeled antibody-analyte complex will migrate to the test zone. If the antibody in the solubilizing reagent is unlabeled, the assay device consists of an antibody-labeling zone. The antibody-analyte complex is labeled by the antibody-labeling reagent at the antibody labeling zone before the solution migrates to the test zone. In either case, the antibody at the test zone is a labeled antibody and the antibody-analyte complex is a labeled antibody-analyte complex.

In a competitive chromatographic assay, the labeled antibody binds to the binding reagent at the test zone, and the labeled antibody-analyte complex flows through the test zone. As a result, the tracer signal at the test zone is inversely proportional to the concentration of analyte in the sample solution. The presence or quantity of the analyte in the sample solution is determined by measuring the tracer signal at the test zone.

In a sandwich chromatographic assay, the labeled antibody flows through the test zone and the labeled antibody-analyte complex binds to the binding reagent of the test zone. At the end, the tracer signal at the test zone is proportional to the concentration of the analyte in the assay sample. The presence or quantity of the analyte in

the sample solution is determined by measuring the tracer signal at the test zone.

Example 1: Moving of Fluorescent dye labeled THC on Fiberglass Membrane:

5 Δ^9 -Tetrahydrocannabinol was labeled with Cy5 dye. The THC moiety of the labeled compound is hydrophobic. Our study revealed that the THC-Cy5 dissolved in 10mM PBS is easily absorbed onto the surface of plastic or glass containers. Fiberglass or porous polyester membrane
10 materials have large surface areas, which can absorb even more THC-Cy5.

A. Preparation of THC-Cy5 Solutions:

Control sample solution is 10mM PBS containing 10nM of THC-Cy5, 0.2% BSA, and 0.5 μ g/ml mouse IgG. A test sample
15 solution is 10mM PBS containing 10nM of THC-Cy5, 0.2% BSA, and 0.5 μ g/ml of mouse anti-THC monoclonal antibody.

B. Preparation of fiberglass and porous polyester membrane strips:

1mm x 8mm x 50mm fiberglass and 1mm x 8mm x 50 mm
20 polyester membrane were laid on 8 mm x 30 mm vinyl strips separately.

C. Migration of THC-Cy5 on fiberglass and polyester membrane materials:

10 μ l of THC-Cy5 control/test sample solution was spiked
25 in the center of each membrane strip at the point that is 15mm to one end (end A) of the strip. 5 drops of 0.2% BSA-PBS solution was gradually applied onto each strip from end A. When the solution migrates from end A to the other end, end B, the strips were scanned with a fluorescence scanner.

30 Result:

On the control strips fluorescence was only detected at the sampling area (7mm-15mm from end A) which suggests that THC-Cy5 compound was absorbed on the membrane materials. On the test strips 80% (75% - 92%) of fluorescence was read at

the half strip of end B. The result shows that the mouse anti-THC monoclonal antibody prevents THC-Cy5 from being adsorbed by the porous membrane materials.

5 Example 2: Chromatographic immunoassay of L-Δ9-carboxy-tetrahydrocannabinol (THCA) in urine.

A. Materials

L-Δ9-THCA from Research Triangle, Institute, Cat# 5754-5 Id, mouse anti-THC monoclonal antibody from Fitzgerald Industries International, Inc, THC-BSA conjugate from LifePoint, Inc. #33, fiberglass and nitrocellulose membrane from Schleicher & Schuell, Inc. were used in this study. All other chemicals were purchased from Sigma-Aldrich Corp.

15 B. Preparation of the Chromatographic Immunoassay Strip for the Detection of THCA

A 5 x 30 mm plastic backed nitrocellulose strip and a 5 x 25 mm Whatman filter paper strip was aligned together on a 5 x 53 mm one-side glued vinyl strip. The plastic backed nitrocellulose strip and the filter paper have a 2mm overlap with each other.

A line of approximately 0.2μg of THC-BSA conjugate was placed on the nitrocellulose membrane at the point approximately 10 mm from the overlap area,

C. Preparation of the soluble assay reagent

25 1 ml of colloidal gold solution (particle size: 20-40μm) was adjusted to pH7.5. 0.02 mg of mouse anti-THC antibody was mixed with the colloidal gold solution. 0.1 ml of 0.01 mole/L sodium phosphate buffered saline containing 1% bovine serum albumin (BSA) was added into the antibody-gold solution. The solution was centrifuged at 8000g for 20 minutes and the precipitated antibody-gold sol conjugate was recovered after carefully removing the supernatant from the centrifuge tube. The antibody-gold sol conjugate was re-suspended in 1 ml of 0.01 mole/L sodium phosphate
30 buffered saline containing 0.1% BSA, 1% trehalose, and 0.05%

Tween 20. 0.01 ml aliquots of the antibody-gold sol conjugate solution were dispensed into 2 ml glass vials and freeze-dried.

D. Preparation of THC-urine samples

5 1- Δ^9 -THCA was spiked into 1 ml urine (from a healthy volunteer) aliquots to provide different drug concentrations in the samples.

E. Chromatographic assay of L- Δ^9 -THCA in urine

10 0.2 ml of urine sample was added into a glass vial having freeze-dried antibody-gold sol conjugate. The antibody-gold sol conjugate was reconstituted by vortex mixing. The nitrocellulose end of a chromatographic immunoassay strip was dipped into the glass vial containing urine sample mixed with antibody-gold sol conjugate.

15 The assay result was read after 10 minutes. The presence of a red line at the test area of the assay strip was interpreted as a negative test result. The absence of red line at the test area of the assay strip was interpreted as a positive test result.

20 Result

All ten samples spiked with 0-2ng/ml THCA tested negative, and all ten samples spiked with >4.0ng/ml THCA tested positive.

25 Example 3: Chromatographic immunoassay of Δ^9 -tetrahydrocannabinol (THC)

Solutions of varied THC concentrations were made by diluting THC into 0.2% BSA-PBS solution. The same device and protocol for testing THCA in urine was used in testing THC solutions.

30 Result:

All ten samples spiked with 0-15ng/ml of THC tested negative, and all ten samples spiked with > 25ng/ml of THC tested positive.

Example 4A. Materials

L- Δ^9 -THCA from Research Triangle, Institute, Cat# 5754-5 Id, Goat anti-mouse IgG antibody from Sigma; THC-BSA conjugate from LifePoint, Inc., and #33 fiberglass and nitrocellulose membrane from Schleicher & Schuell, Inc were used. All other chemicals used in this study were purchased from Sigma-Aldrich Corp.

B. Preparation of the antibody labeling reagent

1 ml of colloidal gold solution (particle size: 20-40 um) was adjusted to pH9. 0.02 mg of goat anti-mouse IgG antibody was mixed with the colloidal gold solution. 0.1 ml of 0.01 mole/L sodium phosphate buffered saline containing 1% BSA was added into the antibody-gold solution. The solution was centrifuged at 8000g for 20 minutes and the precipitated antibody-gold sol conjugate was re-suspended in 1 ml of 0.01 mole/L sodium phosphate buffered saline containing 0.1% BSA, 1% trehalose, and 0.05% Tween 20.

C. Preparation of the chromatographic immunoassay strip for the detection of THC:

A 5 x 30 mm plastic backed nitrocellulose strip was positioned on the middle area of a 5 x 76 mm one-sided glued vinyl strip. A 5 x 25 Whatman filter paper strip and a 25 mm #33 fiberglass strip were separately positioned on the ends of the vinyl strip. Both the filter paper and the fiberglass have 2 mm overlapping with the nitrocellulose strip. 10 ul of goat anti-mouse IgG antibody-gold sol conjugate solution was applied at one spot of the fiberglass 5 mm from the nitrocellulose-fiberglass overlap area.

A line of approximately 0.2 ug of THC-BSA conjugate was placed on the nitrocellulose membrane at the point approximately 10 mm from the nitrocellulose-filter paper overlap area.

D. Preparation of the soluble assay reagent

15 ul of 0.01M sodium phosphate buffered saline containing 0.01 ug/ml of mouse anti-THC antibody, 0.2% BSA,

and 0.1% Tween 20 was added into each 2.5 ml polypropylene vials as shown in figure 3. The solution was freeze dried.

E. Chromatographic assay of 1- Δ 9 - THCA in urine:

0.2 ml of urine sample was added into a polypropylene vial containing mouse anti-THC antibody solution. The antibody solution was reconstituted by vortex mixing. The nitrocellulose end of a chromatographic immunoassay strip having an antibody labeling zone was dipped into the plastic container containing the urine sample mixed with the antibody solution.

The assay result was read after 10 minutes. The presence of a red line at the test area of the assay strip was interpreted as a negative test result. The absence of a red line at the test area of the assay strip was interpreted as a positive test result.

Result

All ten samples spiked with 0-5ng/ml of L- Δ 9-THCA tested negative, and all ten samples spiked with > 10ng/ml of L- Δ 9-THCA tested positive.

Claims

1. A method of determining the presence and/or quantity of a hydrophobic analyte in a biological solution suspected of containing the analyte comprising the steps of:

5 (a) selecting a labeled specific first antibody capable of binding to the hydrophobic analyte in the solution suspected of containing said analyte and blocking the hydrophobic regions of the analyte;

10 (b) adding said labeled specific first antibody to the solution suspected of containing the analyte, said antibody binding to the analyte to form an analyte-antibody complex;

(c) introducing said solution containing the analyte-antibody complex to a solid matrix;

15 (d) flowing the solution containing the analyte-antibody complex along the solid matrix, such that the analyte-antibody complex with the blocked hydrophobic regions of the analyte prevents the analyte from adsorbing to the solid matrix, and wherein said solid matrix has an
20 immobilized specific second antibody to the analyte, said second antibody thus forming a labeled antibody-analyte-second antibody complex; and

(e) detecting the label to determine the concentration and/or presence of the analyte in the
25 solution.

2. A method in accordance with claim 1 wherein said solution containing the analyte-antibody complex is introduced to a sample introduction portion of the solid matrix.

30 3. A method in accordance with claim 2 wherein the labeled analyte-antibody complex is detected downstream from the solution introduction portion of the solid matrix.

4. A method in accordance with claim 1 wherein the labeled analyte-antibody complex is detected within a test zone, said test zone containing the immobilized second specific antibody to the analyte.

5 5. A method in accordance with claim 1 wherein the test solution is saliva.

6. A method in accordance with claim 1 wherein the test solution is urine.

7. A method in accordance with claim 1 wherein the
10 solid matrix is made of a material selected from a group consisting of cellulose, fiberglass, nylon and polyester.

8. A method in accordance with claim 1 wherein the first antibody is labeled with a labeling reagent chosen from the group consisting of fluorescent, chemiluminescent,
15 and bioluminescent labels.

9. A method in accordance with claim 1 wherein the first antibody is labeled with a labeling reagent chosen from the group consisting of radioactive isotopes, enzymes, and particular color particles.

20 10. A method in accordance with claim 9 wherein said particular color particles include colloidal gold, colloidal silver, carbon black, and latex beads.

11. A method of determining the presence and/or quantity of a hydrophobic analyte in a biological solution
25 suspected of containing the analyte comprising the steps of:

(a) selecting a labeled specific antibody capable of binding to the analyte and blocking the hydrophobic regions of the analyte;

(b) adding said specific labeled antibody to the solution suspected of containing the analyte, said antibody binding to the analyte to form an analyte-antibody complex;

(c) introducing said solution containing the
5 analyte-antibody complex to a solid matrix;

(d) flowing the solution containing the analyte-antibody complex along the solid matrix such that the analyte-antibody complex with the blocked hydrophobic regions of the analyte prevents the analyte from adsorbing
10 to the solid matrix, and wherein said matrix has an analyte or an analog of the analyte, said analyte capable of binding to the labeled antibody; and

(e) detecting the label to determine the concentration and/or presence of the analyte in the
15 solution.

12. A method in accordance with claim 11 wherein said solution containing the analyte-antibody complex is introduced to a sample introduction portion of the solid matrix.

20 13. A method in accordance with claim 11 wherein the labeled analyte-antibody complex is detected downstream from the solution introduction portion of the solid matrix.

14. A method in accordance with claim 11 wherein said hydrophobic analyte in the test solution is THC.

25 15. A method in accordance with claim 11 wherein the test solution is saliva.

16. A method in accordance with claim 11 wherein the test solution is urine.

17. A method in accordance with claim 11 wherein the solid matrix is made of a material selected from a group consisting of cellulose, fiberglass, nylon and polyester.

18. A method in accordance with claim 11 wherein the
5 first antibody is labeled with a labeling reagent chosen from the group consisting of fluorescent, chemiluminescent and bioluminescent labels.

19. A method in accordance with claim 11 wherein the
10 first antibody is labeled with a labeling reagent chosen from the group consisting of radioactive isotopes, enzymes, and particular color particles.

20. A method in accordance with claim 19 wherein said particular color particles include colloidal gold, colloidal silver, carbon black, and latex beads.

15 21. A method of determining the presence and/or quantity of a hydrophobic analyte in a biological solution suspected of containing the analyte comprising the steps of:

(a) selecting a specific antibody capable of
binding to the hydrophobic analyte in the solution suspected
20 of containing said analyte and blocking the hydrophobic regions of the analyte;

(b) adding said specific antibody to the solution suspected of containing the analyte, said antibody binding to the analyte to form an analyte-antibody complex;

25 (c) introducing said solution containing the analyte-antibody complex to a solid matrix;

(d) flowing the solution containing the analyte-antibody complex along the solid matrix such that the analyte-antibody complex with the blocked hydrophobic
30 regions of the analyte prevents the analyte from adsorbing to the solid matrix, and wherein said solid matrix contains a labeling reagent capable of binding and labeling the

antibody, wherein said labeling reagent is movably supported in the antibody-labeling zone;

(e) flowing the labeled antibody-analyte complex along the solid matrix, wherein said solid matrix further
5 contains an analyte or an analogue of said analyte immobilized on the solid matrix, said immobilized analyte or analogue of said analyte capable of binding to the antibody to form an-analyte-antibody complex; and

(f) detecting the label to determine the
10 concentration and/or presence of the analyte in the solution.

22. A method in accordance with claim 21 wherein the labeling reagent is a binding protein of the antibody conjugated with a label.

15 23. A method in accordance with claim 21 wherein said analyte is THC.

24. A method in accordance with claim 21 wherein the solid matrix is composed of a material selected from the group consisting of cellulose, fiberglass, nylon and
20 polyester.

25. An immunoassay device for determining the presence and/or quantity of a hydrophobic analyte in a biological solution suspected of containing the analyte comprising:

(a) a chromatographic assay strip having a sample
25 introduction zone, a test zone, and an absorption zone;

(b) a solubilizing assay reagent having a selected movable labeled specific antibody to the hydrophobic analyte in the biological solution, wherein said selected antibody is capable of binding to the analyte to
30 form an antibody-analyte complex and blocking the hydrophobic regions of the analyte, and wherein said solubilizing reagent is added to the biological solution

suspected of containing the analyte and flowed along the introduction zone to the test zone;

(c) an analyte or an analogue of said analyte immobilized in the test zone of the chromatographic assay strip, wherein said analyte or analogue of the analyte is capable of binding to the antibody not bound with the sample analyte, such that detection of the label in the test zone shows the presence and/or concentration of the analyte; and

(d) wherein the absorption zone is capable of holding the unbound reagent flowing from the test zone to the absorption zone.

26. An immunoassay device in accordance with claim 25 wherein the hydrophobic analyte is THC.

27. An immunoassay device in accordance with claim 25 wherein the chromatographic strip is made of a material selected from the group consisting of cellulose, fiberglass, nylon, and polyester.

28. An immunoassay device for determining the presence and/or quantity of a hydrophobic analyte in a biological solution suspected of containing the analyte comprising:

(a) a chromatographic assay strip, said strip contains a sample receiving zone, a test zone and an absorption zone;

(b) a solubilizing assay reagent having a selected labeled antibody to the hydrophobic analyte in the biological solution, wherein said selected labeled antibody is capable of binding to the analyte to form an antibody-analyte complex and blocking the hydrophobic regions of the analyte, and wherein said solubilizing reagent is added to the biological solution suspected of containing the analyte and flowed along the introduction zone to the test zone;

(c) a second antibody to the analyte immobilized in the test zone of the chromatographic strip, said second

antibody capable of binding to the analyte in the analyte-antibody complex and forming a sandwich, such that detection of the label in the test zone shows the presence and/or concentration of the analyte.

5 29. An immunoassay device in accordance with claim 28 wherein the chromatographic strip is made of a material selected from the group consisting of cellulose, fiberglass, nylon, and polyester.

10 30. An immunoassay device in accordance with claim 28 wherein the absorption zone is capable of holding unbound sample flowing from the test zone to the absorption zone.

31. An immunoassay device for determining the presence and/or quantity of a hydrophobic analyte in a biological solution suspected of containing the analyte comprising:

15 (a) a chromatographic assay strip, said strip contains a sample receiving zone, an antibody-labeling zone, a test zone and an absorption zone;

20 (b) a solubilizing assay reagent having a selected specific antibody to the hydrophobic analyte in the biological solution, wherein said antibody is capable of binding to the analyte to form an antibody-analyte complex and blocking the hydrophobic regions of the analyte, and wherein said solubilizing reagent is added to the biological solution suspected of containing the analyte and flowed
25 along the introduction zone to the test zone;

(c) an antibody-labeling reagent located in the antibody-labeling zone;

30 (d) a binding reagent comprising an immobilized analyte or an analogue of the analyte immobilized in the test zone of the chromatographic strip, said binding reagent capable of binding to an antibody unbound by the sample analyte to form an antibody-analyte complex, such that

detection of the label in the test zone shows the presence and/or concentration of the analyte.

32. An immunoassay device in accordance with claim 31 wherein the antibody-labeling zone is located downstream
5 from the sample addition zone, and upstream from the test zone.

33. An immunoassay device in accordance with claim 31 wherein the chromatographic strip is made of a material selected from the group consisting of cellulose, fiberglass,
10 nylon, and polyester.

34. A chromatographic immunoassay device in accordance with claim 31 wherein the absorption zone is capable of holding unbound sample flowing from the test zone to the absorption zone.

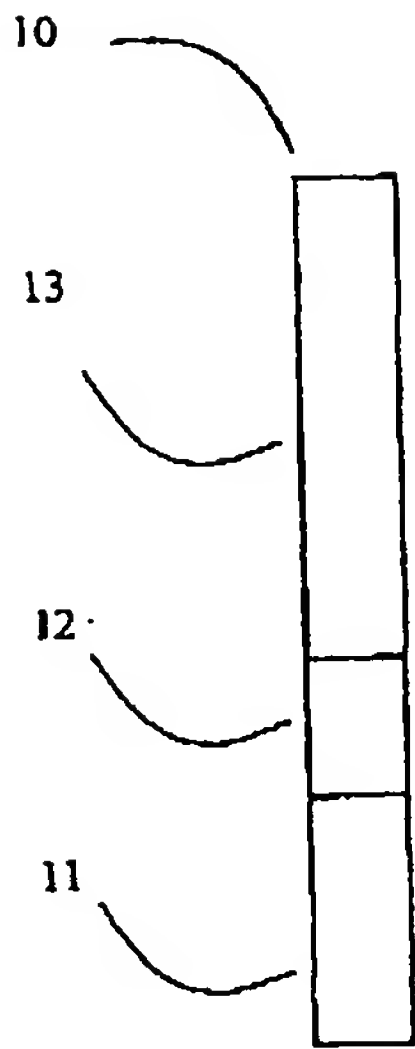


Fig 1

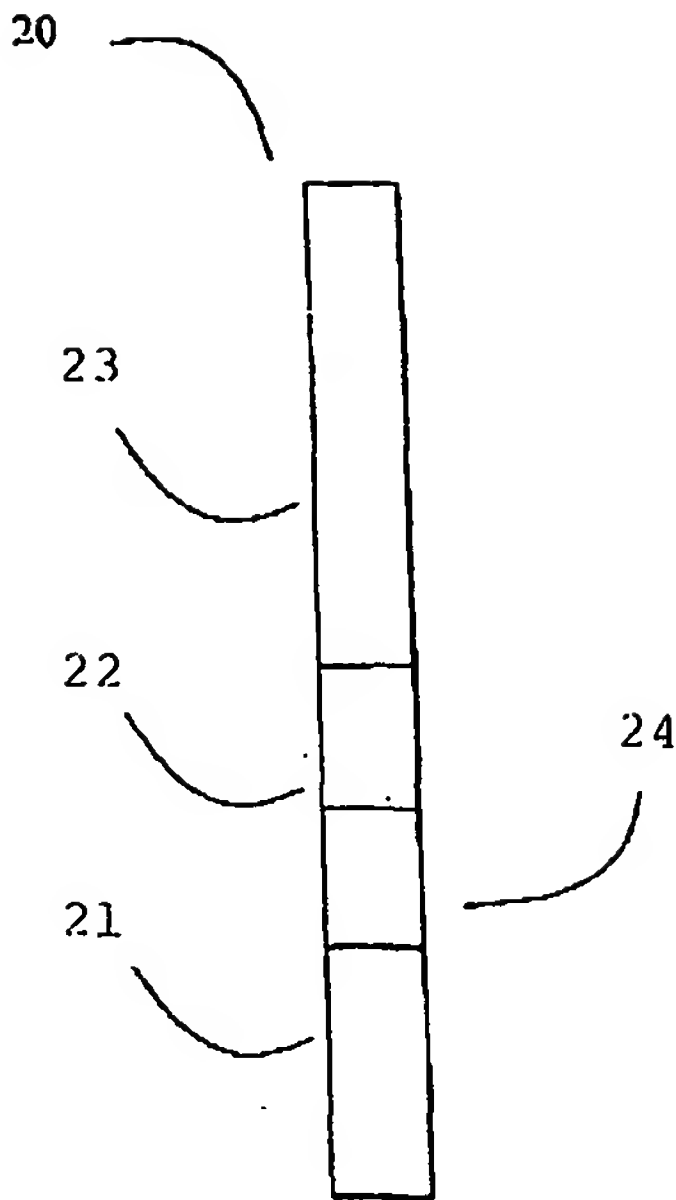


Fig 2

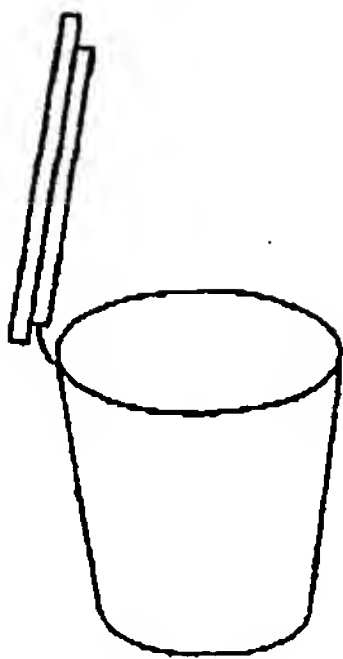


Fig 3